gested that, prior to completion of the translocation, the ribosome oscillates between three states characterized by three configurations of the tRNAs: the classical state (A/A and P/P), the hybrid state (A/P and P/E), and a previously unidentified hybrid state (A/A and P/E) in which the A and P site tRNAs have moved independently (Munro et al., 2007). Another study suggested that the spontaneous motions of the tRNAs are coupled with overall conformational changes of the ribosome (Ermolenko et al., 2007). Using cryo-electron microscopy, we collected over 200,000 projection images of ribosomal pre-translocational complexes carrying a single mutation on the P-loop (G2252C; see Munro et al., 2007). By applying classification to the projection images, we discovered two intrinsic conformations of the complex, one with the ratchet motion and the other without. This finding provides a structural confirmation for the idea that the ratchet motion is encoded in the ribosome architecture itself (Tama et al., 2003), that it can occur spontaneously, and that it provides the dynamics underlying mRNA-tRNA translocation (Frank et al., 2007).

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DNA, RNA Structure & Conformation

281-Pos High-Throughput Fluorescence Method to Determine Thermodynamic Parameters of Nucleic Acids

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Board B114

The nearest-neighbor model provides accurate predictions of stability for nucleic acid duplexes. These predictions are important for design of many biological applications. The model parameters have been traditionally obtained by ultraviolet spectroscopic and calorimetric methods. Since these techniques are low-throughput, thermodynamic parameters have yet to be determined for many significant chemical modifications (e.g., locked nucleic acids, phosphorothioate DNAs, 2′-O-methyl RNAs). Therefore, the effects of these modifications on duplex stability cannot be accurately predicted. We employed dyes and quenchers attached to nucleic acids, so that duplex melting transitions were accompanied by changes in fluorescence. Melting profiles were measured simultaneously for hundreds of duplex DNA samples using modified real-time PCR systems. Various designs of oligonucleotides, dyes and quenchers (FAM, HEX, TET, ROX, Cy3, Cy5, MAX, Rhodamine green, Texas Red, TAMRA, Tye563, Tye665, Alexa Fluor 488, Alexa Fluor 546, Black Hole and Iowa Black Quenchers) were studied. Their ability to monitor duplex denaturation and provide accurate values of enthalpies, entropies and free energies was characterized. The fluorescence method was then used to determine the thermodynamic parameters for chimeric LNA-DNA duplex oligomers.

This project was supported by grant R43GM081959 from the NIH.

282-Pos Electromechanical switching of aptamers

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Board B115

Electrode-immobilized, fluorescently labeled DNA aptamers for thrombin can be stimulated electromechanically using alternating electrical fields. Their motion can be detected optically using energy transfer between fluorophore and the metallic electrode. In the absence of protein ligands, the motion of the aptamers follows the charging response of the electrical double-layer, leading to relaxation-type behavior at kHz frequencies. If thrombin is bound to the aptamers, a second relaxation process appears at much lower frequencies. The motion of the aptamer-protein complex seems to be strongly damped, which may be attributed to hydrodynamic effects or the interaction with neighboring aptamer strands or aptamer-protein complexes. The slow relaxation process can be utilized for sensitive detection of thrombin.

283-Pos Biophysical Study of Native and Locked Nucleic Acids

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Integrated DNA Technologies, Coralville, IA, USA.

Board B116

Locked nucleic acids (LNA) are bicyclic ribonucleotide analogs that show enhanced affinity and specificity against nucleic acid targets. To improve understanding of these effects, the dependence of thermodynamic stability on solution composition was studied for a series of DNA-DNA, RNA-RNA, RNA-DNA and LNA-DNA chimeric duplexes using UV spectroscopic and calorimetric melting experiments. We found that LNA nucleotides do not change the amount of sodium counterions released when duplexes denature. Therefore, the model that predicts effects of sodium ions on stability of native DNA duplex oligomers is also accurate for LNA-DNA chimeric duplexes. Osmotic stressing technique demonstrated that LNA modifications decrease the amount of water molecules released upon duplex melting substantially.

284-Pos On the Importance of the N7 of Guanine for the Formation of DNA Frayed Wires

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Board B117

Oligodeoxyribonucleotides comprised of long, consecutive, terminal guanines, ($d(N_xG_y)$ or $d(G_yN_x)$, $x \geq 5$, $y \geq 12$), self-aggregate to form a polydisperse set of multi-stranded structures termed DNA frayed wires (FW). These structures exhibit extreme thermostability and resistance to chemical denaturants and are held together through

guanine-guanine self interactions. Previously, our group's methylation protection data suggested that N7 of guanine was not taking part in any hydrogen bonding. Therefore, it was assumed that FW have a different hydrogen-bonding pattern from G-quadruplexes, which are of the Hoogsteen type, that involve the N7 of guanine. We employed the modified nucleotide pyrazolo[3,4-*d*]pyrimidine (PPG) into a sequence that readily forms FW, $d(A_{15}G_{15})$. We report that $d(A_{15}z^{8}c^{7}G_{15})$ did not form any higher-ordered structures in the presence of various cations and incubation times of up to 10 days which highlights the importance of N7 of guanine for the formation of FW. Furthermore, we show that FW composed of four parent strands, $d(A_{15}G_{15})$, are the most stable structures despite the presence of assemblies comprised of a larger number of aggregated strands. This is thought to be the result of an assembly that arises from the most in-register compilation of strands.

285-Pos Static And Dynamic Flexibility Of Single-stranded Oligonucleotides

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Board B118

Biopolymers, such as single-stranded DNA (ssDNA), are often described as semiflexible polymers or worm-like chains. We investigated static and dynamic flexibility of homogeneous ssDNA (polythymine), a model polyelectrolyte, in various conditions of ionic strength.

Using fluorescence correlation spectroscopy we found that the hydrodynamic radius Rh scales with contour length according to a power law with an exponent between 0.5 and 0.7 depending on ionic strength I. For polythymine with 100 residues we found that the persistence length Lp scales as Lp ~ Îm with m = (−0.22±0.01), assuming that Rh is proportional to the square root of Lp.

For comparison we performed Molecular Dynamics (MD) simulations with a force field that accounts for short-range interactions in vacuum, and determined the characteristic polymer properties end-to-end distance R, radius of gyration S, and persistence length Lp of various labeled and non-labeled polythymine derivatives. We found excellent agreement for the length dependences of simulated S and experimental Rh measured at 100 mM NaCl, revealing that electrostatic interactions are completely shielded in aqueous solution with such ionic strength.

These results provide a benchmark for theories and MD simulations describing the influence of electrostatic interactions on polyelectrolyte properties, and thus help to develop a complete and accurate description of ssDNA.

286-Pos Probing Stereospecific Environment In DNA Using Site-Directed Spin Labeling and MD Simulations

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Board B119

Site-directed spin labeling utilizes a stable nitroxide radical to obtain site-specific structural and dynamic information on a macromolecule via EPR spectroscopy. We have developed a scheme for covalent attachment of nitroxide species, such as 1-oxyl-2,2,5,5-tetramethylpyrroline (R5) and 1-oxyl-4-bromo-2,2,5,5-tetramethylpyrroline (R5a) to chemically substituted phosphorothioate positions at the DNA/RNA backbone. It has been previously shown that the EPR spectra of R5 and R5a report site-specific dynamic features within a B-DNA duplex. However, solid-phase chemical synthesis introduces two phosphorothioates diastereomers (Rp and Sp) in ~ 50/50 ratio, and the nitroxide attached to those diastereomeric forms may have different EPR spectral signatures due to differences in the macromolecule environment. We have obtained X-band EPR spectra of R5 and R5a attached at pure Rp and Sp diastereomers at the center of a B-form DNA duplex. The observed EPR spectra consistently show small differences in the nitroxide mobility. These experimental data is being correlated with the results from Molecular Dynamic simulations, which predicted that the Rp label at this position has a propensity for interaction with the methyl group of the 3′ thymine nucleotide. Our results demonstrate the ability to reveal detailed features of DNA environment using a combination of site-directed spin labeling and MD simulation.

287-Pos Monte Carlo Simulation of Transcriptional Regulation in Linear DNA of Eukaryotes

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Board B120

It is well established that gene expression in eukaryotes is controlled by sequence-dependent binding of trans-acting proteins to regulatory elements like promoters or enhancers. A less well understood level of gene regulation is governed by the various conformation and properties of DNA or chromatin. Recently, new viewpoints of studies on gene expression which show the relationship between conformation and gene expression in eukaryotes have been appeared. First, long-range control of gene expression can be mediated through direct physical interactions between promoters and enhancers[1,2]. Second, structural change of DNA, enclosure of an enhancer in a DNA loop, can block activation of gene expression[3]. However, physical origins of such DNA-structure-dependent gene regulation is not clear. How is structural change of DNA linked to gene expression? What properties of DNA, chain stiffness and electrostatic interaction, etc., do affect gene expression? To address these issues, we perform Metropolis Monte Carlo simulations for linear DNA using a worm-like chain DNA model which takes into account gene expression in eukaryotes. In this presentation, we argue how conformation and properties of DNA affect gene expression. Detailed results and discussion about physical origin of the DNA-structure-dependent gene regulation will be given in the presentation.

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288-Pos Nanopore Transduction Analysis of Transcription Factor Binding, HIV DNA Terminus Dynamics, and HIV Integrase Binding to Host DNA

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Board B121

X-ray crystallographic studies have guided our understanding of DNA structure for almost a century and protein structure for almost 60 years. Precise X-ray results are prohibitively difficult to obtain for larger proteins, membrane proteins, and for resolving multi-component biomolecular interactions. With a nanopore detector, however, it may be possible to examine many such systems in a novel way, while still in solution and on a single molecule basis. DNA-protein binding is studied by monitoring transduction-induced changes in the blockade patterns produced by a Y-shaped dsDNA molecule inserted within the nanopore detector. The binding targets of the channel-captured molecules are well away from the channel-captured region, and include non-terminal and terminal regions of dsDNA. The first set of experiments continues work to examine binding between a TATA box receptor, located near the terminus of one arm of our three-way dsDNA junction, and TBP. In a second series of experiments, we examine binding between HIV's DNA integrase and a blunt-ended dsDNA terminus encoded with the HIV highly conserved DNA's terminus. The nanopore transduction experimental results provide information-rich material for our cheminformatics data analysis approach. Synthetic transcription factors (STFs) offer a powerful new therapeutic against Cancer, AIDS, and genetic disease. Currently, 10% of drugs are of this type, including salicylate and tamoxifen. Using nanopore-detection approaches it may be possible to greatly accelerate drug-discovery efforts involving STFs.

289-Pos Conformational Stability of Fluorescently Labeled Human Telomeric G-quadruplexed DNA Sequences

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Board B122

Telomeric guanine-rich single stranded DNA located at the end of chromosomes forms a four-stranded intramolecular G-quadruplex in vitro. This structure can inhibit the activity of the enzyme telomerase, which is found in tumor cells, and is thus a target for potential cancer therapeutics. We are interested in the conformational stability of the quadruplex structure and its effect on the binding of quadruplex interactive agents (QIAs). To this aim, we have designed a number of fluorescent analogs of the HT4 quadruplex sequence through incorporation of the fluorophore 6-methyl-8-(2-deoxy-D-ribofuranosyl) isoxanthopterin (6MI) at guanine-tetrad positions:G1, G5, G9, G11, and (G5,11). Thermal studies confirm that with increasing KCl, the formation of quadruplex structures is promoted. In addition, a decrease in the fluorescence intensity of 6MI-labeled DNA was observed with quadruplex formation, dependent on the position of the label suggesting altered conformational stabilities within the quadruplex structure. As expected, the fluorescent telomeric sequences exhibit a lower melting temperature compared with the parent sequence. We have utilized N-methyl mesoporphyrin IX (NMM), a porphyrin known to selectively bind to the quadruplex structure via intercalation as a tool to spectroscopically investigate the integrity of the fluorescent quadruplex structures.

290-Pos Exploring The Characteristics Of A Complex DNA Aptamer Using The Fluorescent Pteridine Nucleoside Analog, 6MI, Incorporated At Various Positions In The Sequence

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Board B123

The 37-base DNA aptamer, D17.4, is known to bind with nM affinity to human immunoglobulin E, IgE, in a highly specific manner and is predicted to have the structural elements of a stem, a single base bulge and a hairpin loop. We have selected this molecule as a model system for probing with the fluorescent pteridine nucleotide analog, 6MI. We compare steady state anisotropies of 3MI and 6MI, each of which is directly incorporated into the 5′ end through a deoxyribose linkage, with that of Texas red, attached to the 5′ terminus of the sequence through a 21-carbon linker. Steady state anisotropies of 3MI and 6MI were 0.013 and 0.018 respectively, while that of Texas red was 0.16. Results for the pteridines suggest rapid segmental motion, possibly a feature of this site. For Texas red, the linker attachment likely permits most of the probe to "fold back" and associate with the rigid annealed sequence, providing anisotropy values determined mostly by the molecule's overall motion. 6MI was also incorporated into three additional sites within the aptamer: 9, 16, and 29 positions in from the 5′ end. Steady-state anisotropies of 6MI at these positions range from 0.11 to 0.08. Time resolved

anisotropies should reveal whether these steady-state differences represent actual changes in the motion of the DNA aptamer, or if they are the result of changes in lifetimes and fluorescence intensities. We will discuss how use of *multiple* reporter sites for target-aptamer binding can allow one not only to select sites with high signal-to-noise and affinity- reporting fidelity, but also to observe more than one mode of binding.

291-Pos DNA Cages

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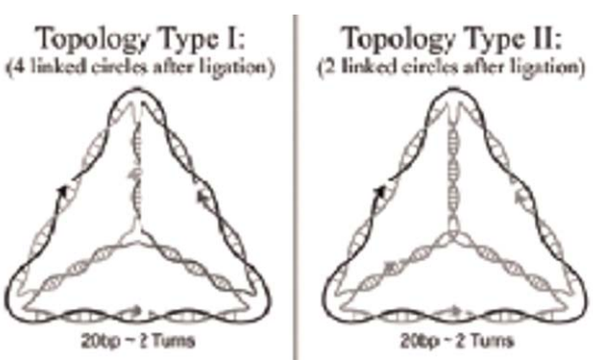
Board B124

DNA polyhedra can be formed in single-step self-assembly from synthetic oligonucleotides. The edges of these polyhedra are DNA double helices and the vertices are 3- or 4-arm junctions. We have explored the formation of regular and irregular DNA polyhedra of different sizes, including tetrahedra, octahedra and a trigonal bipyramid.

We can design the base sequence of each oligonucleotide, such that they will hybridise to form the desired three-dimensional structure. The assembled structure contains nicks where the 5′ and 3′ ends of the oligonucleotides meet. These nicks can be covalently closed by DNA ligase resulting in polyhedra that consist entirely of closed circular loops of DNA: either four or two interlinked loops are formed, depending on how we have chosen to thread the oligonucleotides along the tetrahedron structure. We have analysed the formation of tetrahedra with different topologies.

DNA polyhedra could serve as cages for other mole-ules. We have demontrated the encapsulation of a single protein molecule inside a DNA tetrahedron and present strategies for assembling cages around proteins using non-covalent interactions.



292-Pos Electrophoretic Mobility Is a Reporter of Hairpin Structure in Single-Stranded DNA Oligomers

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University of Iowa, Iowa City, IA, USA.

Board B125

The electrophoretic mobilities of single-stranded DNA oligomers containing 26 nucleotides, and the corresponding duplexes, have been measured in polyacrylamide gels and in free solution. The mobilities observed for the dsDNAs were essentially independent of sequence and base pair composition. However, the mobilities observed for the single-stranded oligomers varied by ±20% in polyacrylamide gels and ±10% in free solution. The mobilities of the single-stranded oligomers became equal at elevated temperatures or when urea was added to the solution, suggesting that the variable mobilities observed at 20°C were due to hairpin formation. Thermal melting profiles were measured for eight of the single-stranded oligomers, and compared with the melting temperatures predicted by the structure-prediction algorithm DINAMelt[1]. The observed melting temperatures of four oligomers were reasonably close to the values predicted by DINAMelt. However, three oligomers were random coils at 20°C, instead of hairpins as predicted by DINAMelt. In addition, one oligomer was unusually stable, and melted a temperature ~20° higher than observed for the other hairpins or predicted by DINAMelt. Thermal melting profiles were measured for two oligomers as a function of buffer concentration. The results suggest that the number of cations released upon thermal denaturation of a DNA hairpin depends on the identity of the monovalent cation, most likely because different monovalent cations bind to DNA with different affinities. The combined results indicate that the electrophoretic mobilities observed for single-stranded DNA oligomers reflect the relative populations of hairpin and random coil structures in the conformational ensemble.

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293-Pos B-S Phase Transition in Stretched DNA

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Board B126

With advent of single molecule manipulation techniques, mechanical properties of DNA have been a subject of extensive studies. One striking discovery was a plateau in the force-extension curve of double-stranded DNA (dsDNA) that signifies a transition from a canonical B-DNA structure to an overstretched state. The nature of the overstretched state, however, remains controversial, as it is not clear whether the overstretching plateau reflects a change to a new stable structural state, i.e. the S-ladder DNA, or indicates a local melting of the two DNA strands. To simulate the overstretching transition in dsDNA using all-atom molecular dynamics, we have developed the following computational scheme. A fragment of dsDNA (two helical turns in length) was covalently connected upon itself across the periodic boundary of the simulated system, representing thereby an effectively infinite DNA molecule. The resulting molecule was submerged in electrolyte solution. To induce DNA stretching, a negative pressure was applied in the direction of the DNA helix while a constant pressure of 1 bar was maintained in the

directions perpendicular to the DNA helix. The simulations revealed a hysteresis of the force-extension dependence at the beginning of the plateau and coexistence of elastically stretched and overstretched phases in the plateau region. The base-pairing pattern was not preserved in the overstretched phase. Our results demonstrate that in the overstretching region the DNA structure undergoes a first-order phase transition. Our results also suggest that melting of dsDNA may start at the beginning of the overstretching transition, which is consistent with the thermodynamic analysis of Rouzina and co-workers.

294-Pos Computational Elastic Rod Theory Captures DNA and Protein Flexibility in the Lac-repressor Complex

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Board B127

DNA looping in the Lac-repressor (LacI) system is important to understand both experimentally and theoretically. Several enduring questions remain about its structure and function including the possible role of protein flexibility in looping mechanics. Experimental data suggests concentrated flexibility at the C-terminus that allows deformation from its stress-free 'closed' or 'V' conformation (as defined by the crystal structure) into an 'open' or 'extended' conformation. By contrast, other studies implicate local protein flexibility in the head domain. Here, we extend a rod model previously used to describe DNA deformation to simultaneously model the flexibility of the protein. Our approach is to model the protein as an articulated elastic body (an extended rod) having non-uniform elasticity that accounts for both 'stiff' and 'flexible' protein domains. The model describes the stress-free conformation of the protein by prescribing the stress-free curvature of the associated rod representation. A numerical algorithm is then employed to compute the equilibrium conformations of the entire DNA-LacI complex including major topological features (binding topology, linking number, loop geometry) and elastic energy. We present numerical results showing the influence of operator phasing and protein flexibility on the energy of formation of the wild-type DNA-LacI complex and compare these predictions to established experimental data in the literature. A second study examines the role of protein flexibility for a family of highly curved (designed) sequences that form hyperstable loops.

295-Pos DNA Quadruplex Molecularity

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Board B128

Nucleic acid quadruplexes form a class of unusual structures whose biological significance is increasingly being demonstrated. Several studies have reported on the quadruplex-forming properties of certain guanine-rich sequences with the assumption that the quadruplex formed is unimolecular. Similarly, another sequence that has been assumed to be unimolecular by many researchers (the thrombin binding aptamer, d(GGTTGGTGTGGTTGG)) has recently been described as bimolecular. While these results generally have been based on spectral and gel behavior of the oligonucleotides, unambiguous molecular mass determinations have not been done. In the results presented here, we describe circular dichroism, polyacrylamide gel and equilibrium sedimentation results on several such quadruplex-forming systems in an effort to obtain reliable measures of their molecularity. Our results indicate that in some cases the previously assumed quadruplex molecularity is incorrect. Consequences of this misinterpretation of incomplete experimental characterization of molecular mass are discussed.

This work has been supported by grant GM067607 from the National Insitute of General Medical Sciences, NIH.

296-Pos The Meltable Wormlike Chain

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Board B129

Double-stranded DNA is often tightly bent in vivo, and indeed the details of DNA bending elasticity crucially influence many cellular processes, such as transcriptional regulation. We provide a rigorous statistical mechanical treatment of the meltable wormlike chain (WLC), a discrete WLC model with thermally activated melts, and show that it makes qualitatively different predictions for cyclization experiments than previous treatments. Furthermore, we show that this thermodynamically sound meltable WLC model provides insights into novel experiments that plumb a wide range of different aspects of DNA bending elasticity.

297-Pos Investigating the Effects of Flanking Sequences on the Structure and Dynamics of DNA Backbones

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Board B130

FTIR and ^{31}P NMR have been used to determine structural changes and the relative flexibilities conferred by different flanking sequences on DNA binding sites. Our FTIR approach has allowed us to examine unique peaks and subtle changes in the spectra of d

(AAAGAATTCTTT)$_2$, d(TTCGAATTCGAA)$_2$, d(AGAGAATTCTCT)$_2$, d(CACACGTGTG)$_2$, and d(TCCACGTGGA)$_2$, and thereby identify local changes in base-pairing, base-stacking, backbone conformation, glycosidic bond rotation and sugar puckering in the studied sequences. NMR spectra demonstrate changes in both the B_I/B_{II} backbone structure and backbone dynamics as a result of changing the sequences flanking the *Eco*RI (-GAATTC) and CRE (-ACGT-) binding sites. These results highlight the important role that flanking sequences play in protein-DNA recognition events.

298-Pos Initiation and Dynamics of Cruciform Formation in AT-rich Palindromic Sequence by Using a Combined Technique of Single Molecule FRET and Magnetic Tweezers

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Board B131

DNA has various structural polymorphisms such as Z-DNA, G-quadruplex, and cruciform. Among them, DNA cruciform is perhaps the most noteworthy because of its role in DNA replication and mutagenesis. A cruciform formed at inverted repeats has a four-way junction because the inverted repeats on both strands form hairpins. Since transient hairpins during replication can cause errors in DNA replication, inverted repeats are often deleted or inserted, leading to various types of serious mutagenic diseases. Although the mechanism of cruciform formation has been extensively studied in the past, initiation and dynamics of DNA cruciform are not fully understood. To investigate formation and dynamics of the cruciform structure in a short AT-rich palindromic sequence at single molecule level, we developed a combined technique of single molecule FRET and magnetic tweezers, which enables us to observe conformational dynamics of a single DNA molecule in real time under tension and torsion which are two important physical variables on DNA supercoiling and cruciform formation. We observed that DNA molecules displayed cruciform structures only when negatively supercoiled, whereas they remain in B form in the absence of torque. Moreover, we found that even under the same level of applied force and torque, the FRET measurements on DNA cruciform showed multiple FRET states, which suggests that there is heterogeneity in hairpin size. We propose there exist multiple and discrete states in cruciform initiation.

299-Pos Discovery And Characterization Of A Highly Stable Structure In The C-myc Promoter

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Board B132

C-myc is an oncogene whose overexpression is associated with various human cancers. As the transcription of the gene is known to be strongly correlated with the stability of the nuclease hypersensitivity element III (NHE III$_1$), a G/C-rich region located 115 bases upstream from the P1 promoter of the gene, researchers tried to identify the structure of NHE III$_1$ as a potential target for anti-cancer treatments. Based on G/C-richness in the element, and DNA footprinting and circular dichroism measurements, a number of different guanine-quadruplex (GQ) structures in anti-sense strand were proposed, but the debate seems to be settled down by recent NMR studies, which clearly show parallel forms of GQ. However, traditional ensemble measurements, including NMR spectroscopy, are not efficient in identifying structural heterogeneity of biomolecules, especially when high-level of polymorphism is expected as in the NHE III$_1$.; five guanine stretches existing in NHE III$_1$, three runs with four guanines and two runs with three guanines, make a number of different combination for quadruplex formation possible. To address this question, we used single-molecule FRET technique, and, as a result, could identify different conformers of NHE III$_1$, and their relative population. Surprisingly, the main conformer, which comprises ~ 50% of the total population of NHE III$_1$, was not a GQ structure as predicted in previous studies. By doing systematic mutations studies, we showed that the new structure is not any of GQ structures formed by different combination of G stretches. Compared to GQ structures, the formation of the newly discovered structure was not sensitive to either the kind of cations nor their concentration. Considering the importance of NHE III$_1$ as a drug target, further studies are required to identify its structure and biological importance.

300-Pos A Study of DNA Triplex with TT Tight Turn by Native Gel Electrophoresis, CD, And Fluorescence Spectroscopy

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Board B133

Following our previous in depth studies on the role of the apex CC pyrimidine-pyrimidine residues in tight-turn configuration of DNA triplexes (*Biochemistry*, *39*, 12457–12464 (2000), *Biophys. J.*, *82*, 3170 (2002), *Biophys. J.*, *91*, 2552–2563 (2006), *Biophys. Rev. Lett.*, *1*, 215–227 (2006)), we found a second sequence which could make similar tight turns. The sequence contains a TT pyrimidine-pyrimidine motif in a triplex oligo DNA formed by 5′GAGAGA3′ (1) and 5′CTCTC**TT**CTCTC3′ (2, the tight turn shown by bold face letters). We defined "tight turn" as a structure in a DNA where the chain turns without intervening residues. The results obtained from native gel electrophoresis showed that only one band, which corresponded to a length of 18-nucleotidyl units, was observable for the mixture of (1) + (2). Its CD spectrum presented a characteristic triplex band. The FRET has been done by linking a quencher and a fluorescin on the 3′- and 5′-ends of (2), respectively. The thermodynamic parameters were obtained by tritration of (1) into (2) in a

temperature range of 5 to 35 C. At present, we demonstrated that this novel tight turn structure occurs, at least, in triplexes with both CC and TT pyrimidine-pyrimidine motif. The biological significance of the novel DNA structure is under investigation.

301-Pos Modeling the Effects of Chirality on DNA Supercoiling

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Board B134

Elastic rod models of DNA have offered important insight into DNA mechanics over the past decade. The majority of the work done in this area has relied on an isotropic material law. However, in recent years, it has been recognized that the chiral nature of the DNA molecule renders a twist-stretch coupling in the material law that plays an important role in its mechanical response. This coupling is evident in single-molecule experiments that twist DNA under tension. Our approach is to model DNA as an elastic rod with a hemitropic material law that captures this coupling. We present results showing the effect of the hemitropy on buckling of rods under prescribed twist and tension. We use experimental data to predict the twist-stretch coupling for DNA and compare our simulations to published results of single-molecule experiments on DNA supercoiling.

302-Pos Systematic Design of RNA Nanostructures

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RNA structures can possess many structural motifs like junctions, bulges, internal loops and loop-loop interactions. Recently we made a comprehensive set of these structural elements available in an extracted and annotated form as part of the RNAJunction database (http://rnajunction.abcc.ncifcrf.gov).

Here we show how these structural elements can be used for the systematic design of RNA nanostructures. Using a combinatorial scanning approach, we generated in a completely automatic fashion a plethora of computational models of RNA triangles, squares, pentagons, dendrimers and other structures. We demonstrate the relationship between RNA rings, spirals and rod-like structures and show how this approach can be used for designing novel RNA nanostructures.

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303-Pos Structural Transitions in the AR7 Riboswitch Aptamer with Purine Binding Studied by Differential Scanning Calorimetry

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Riboswitches are recently discovered RNA sequences that have been shown to regulate gene expression by undergoing conformational changes as they bind small metabolites. This conformational change affects the transcription or translation process, thus switching the gene either on or off. Our study focuses on the aptamer region of the AR7 riboswitch, which binds purine metabolites and regulates genes involved in purine biosynthesis. Our goal is to correlate these RNA structural changes with metabolite binding to gain further insight into the riboswitch gene regulation mechanism. Differential scanning calorimetry (DSC) was used to thermally unfold the aptamer region of AR7 in the presence of the binding metabolite 2,6-diaminopurine (DAP). Without DAP, only two sequential non-two state transitions were resolved in DSC scans: the first was a tertiary structure unfolding transition followed by a large secondary structure transition. With DAP, the initial tertiary structure with no added DAP was resolved into two transitions, the first corresponding to a tertiary transition independent of DAP concentration and the second corresponding to the binding pocket unfolding transition. As the concentration of DAP was increased, the binding pocket transition shifted to a higher temperature and the unfolding enthalpy increased and remained constant. The AR7-binding metabolites 2-aminopurine, purine, adenine and hypoxanthine showed significantly less affinity for AR7, giving insight into the role amine functional groups play in binding.

304-Pos Characterization of Structural Elements for RNA Nano Design

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We recently developed the RNAJunction database, which contains more than thirteen thousand RNA junctions, bulges, internal loops and loop-loop interactions (http://rnajunction.abcc.ncifcrf.gov) from which we derived clusters of structural elements with identical sequences. We correlated root mean square deviations present in each cluster with the root mean square deviations of the structures minimized with the help of the Amber package, and we found that the structural variations in the clusters are significantly correlated with the structural variations resulting from energy minimizations. We also discovered dependencies between the average structural changes of the RNA structural elements within a cluster and their

topology. Thus we use the information derived from the database clustering to characterize structure elements as potential RNA building blocks. Together with techniques such as molecular dynamics simulations on selected structural motifs, we use this structure variability information to design RNA nano-scale structures. We present examples of the RNA nano-scale modeling process with emphasis on the characterization of the structural motifs used as building units as well as that of the entire structure.

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305-Pos How Large is a Large RNA?

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Single-stranded (ss) RNA molecules are involved in virtually every part of the cell and in a wide variety of roles, e.g., as messenger RNA, transfer RNA, structural RNA, and enzymatic RNA. Furthermore, a large majority of viral genomes are ssRNA molecules, often as long as 10,000 nucleotides (nt) in length. In this work we address the question: what are the 3D sizes of large ssRNA molecules and how do they depend on nucleotide length and sequence? To determine their physical dimensions, we measure the radii of gyration (R_g) and hydrodynamic radii (R_h) of several natural and engineered RNAs whose lengths range from a few hundred to several thousand nucleotides.

We find that for a fixed nucleotide length the 3D sizes of RNAs with different base sequences can vary enormously. For example RNAs of length ~2000nt with roughly the same base composition can vary in R_g and R_h by over 30%. Moreover, by using low ionic-strength buffers to minimize tertiary interactions within the RNA molecule, we demonstrate that the size discrepancies need not arise from specific long range contacts, but instead are a generic property of the secondary structure, which in turn is determined by the nt sequence. Some viral RNAs that self-assemble into spherical protein capsids are presented as highly evolved cases where the primary sequence codes for a relatively compact size and shape. For example, the R_g of a 3000nt viral RNA from CCMV is of the order of that for a 1000nt non-viral sequence.

R_h's are obtained from fluorescence correlation spectroscopy (FCS) measurements and R_g's from small-angle x-ray scattering (SAXS). Pair distribution functions and Monte-Carlo reconstructions derived from SAXS studies are used to illustrate the intrinsic anisotropy of selected molecules and their ramifications for biological processes such as viral capsid assembly.

306-Pos Molecular Ångström Optics: A dynamical view of biomolecular structure

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Using a confocal fluorescence microscope the newly developed multiparameter fluorescence detection (MFD) enables us to simultaneously collect all fluorescence information such as intensity, lifetime, anisotropy in several spectral ranges) from picoseconds to seconds. MFD is applied to perform single-molecule fluorescence-resonance-energy-transfer (FRET) studies on nucleic acids labeled with a fluorescent donor and acceptor dye. Thus, it is possible to circumvent the classical pitfalls of the FRET method in ensemble measurements. These novel FRET-based detection and analysis methodologies allowed us to resolve structural subpopulations with sub-nanometer resolution. Furthermore, direct access to the time trajectories of the different fluorescence parameters is obtained revealing the dynamics of the system. Finally, the construction of more-dimensional frequency histograms of the fluorescence parameters found in the trajectories on the single molecule level and selective analysis of these species (e.g. selective correlation analysis) give detailed view on the molecular energy landscape and the associated molecular structures.

Moreover a probability distribution analysis (PDA) method for calculating a priori histograms of FRET signals is presented taking explicitly crosstalk, stochastic variations and background into account. Histograms for the shot noise limited FRET signal are obtained by finding the mean as the only parameter in a least squares fit. Error analysis suggests an ultimate level of precision in determining separations with FRET of 1% of the Förster radius. The PDA method unambiguously distinguishes between stochastic processes and broadening due to signal heterogeneity. In this way quantitative structural information on various bent and kinked DNA and RNA structures was obtained. Moreover the structural and dynamic properties of the folding intermediate of a Holliday junction could be characterized. Finally single-molecule fluorescence studies on nucleic acid binding proteins will be discussed showing that MFD has developed to a powerful tool for Molecular Ångström Optics.

Membrane Physical Chemistry - I

307-Pos PEO-PPO Block Copolymer Vectors Do Not Interact Directly with DNA but with Lipid Membranes

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